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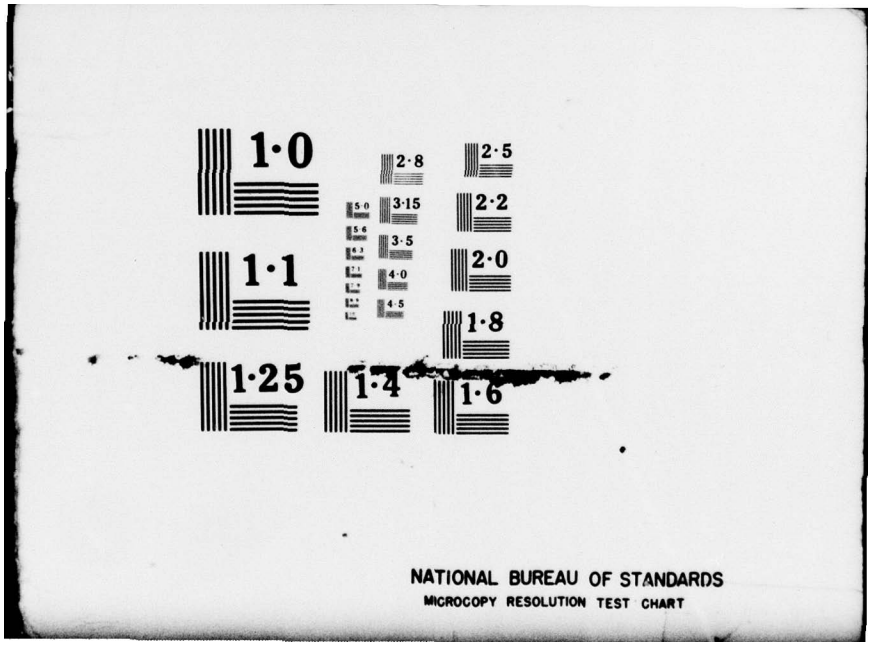
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Chemotherapeutic Studies on Schistosomiasis. (U)

Annual Technical Report
July 1976 - September 1977,

Dr. Aluizio Rosa Prata, M. D.
LTC Myron G. Radke, MSC

October 1977

Supported by

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Washington, D. C. 20314

Contract No. DAMD 17-G-9427

University of Brasilia
70.000 Brasilia, D. F., Brazil

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SUMMARY

The collaborative anti-schistosome drug testing program between the University of Brasilia and the U. S. Army Medical Research Unit (WRAIR) Brasilia continues to screen new chemical compounds for prophylactic and therapeutic activity against schistosomiasis.

The U. S. Army's Anti-schistosome Drug Development Program, Walter Reed Army Institute of Research, provides selected drugs for prophylactic and curative testing in mice infected with schistosomiasis mansoni. The drugs are tested initially for prophylactic activity by the mortality test system which uses mice exposed to 3,000 or more S. mansoni cercariae. Drugs are prepared in peanut oil and administered at 1280 mg/kg to mice in a single subcutaneous inoculation two days after the cercarial exposure. Some selective and all active drugs as detected by the prophylactic test are then tested for curative activity (although the test system is under development) in mice exposed to 200 S. mansoni cercariae, and 30-35 days later, drugs are administered subcutaneously at 100 mg/kg for five consecutive days. Prophylactic drug activity is measured by mouse survival; whereas, curative drug activity is measured by the number of live and/or dead worms found in liver squash preparations from the infected/treated mice.

The laboratory Biomphalaria glabrata snail colony supported a daily inventory of 1,318 S. mansoni snails shedding cercariae. The weekly cercarial collections of five million were more than adequate to infect prophylactic test group mice with 3,000 or more cercariae.

During FY77, a total of 2,096 bottle number drugs were screened by either the prophylactic or the under development curative test system for anti-schistosome activity. In the mortality test for prophylactic activity, 1,310 bottle number drugs were tested at 1280 mg/kg and the test results were: 879 negative, 428 toxic, and 3 active. In the curative test for therapeutic activity, 786 bottle number drugs were screened at 100 mg/kg and the test results were: 704 negative, 41 toxic, 37 unconfirmed active, and 4 active. One discreet drug was active in both the PMT and PCT.

The UnB/USAMRU-Brasilia program will continue to screen selected drugs for prophylactic and curative anti-schistosome activity.

FOREWORD

The research program of the University of Brasilia and the U. S. Army Medical Research Unit (WRAIR) Brasilia is to test drugs for prophylactic and curative activity against schistosomiasis mansoni.

The research project is carried on under the following project and task number:

3M762770A802, Task 00, Work Unit 009

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", DHEW Publication Number (NIH) 73-23; as prepared by the Institute of Laboratory Animal Resources, National Research Council.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

ALUIZIO ROSA PRATA, M. D.
Professor of Tropical Medicine

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BODY OF REPORT

PROJECT NO. DAOB 6525
3M762770A802: Tropical Medicine

TASK NO. 00: Tropical Medicine

WORK UNIT 009: Chemotherapeutic Studies on Schistosomiasis

DESCRIPTION:

It is an appalling fact that schistosomiasis remains one of the few tropical diseases in which there are few drugs to use for treatment. The limited drugs marketed, Niridazole, Hycanthone, and Oxamniquine, give about an 80 percent cure rate but administratively accompanied by some major side-effects. At no time in our medical history have we been so vulnerable to a tropical disease as schistosomiasis; prophylactic drugs are non-existent and curative drugs are limited. Today's global strategic areas are Africa and the Middle East which are hot-beds of schistosomiasis. A major research effort in the anti-schistosome drug development program is being carried out by the U. S. Army Walter Reed Army Institute of Research. The drugs are obtained from the Division of Medicinal Chemistry (WRAIR). These drugs are tested for prophylactic and curative anti-schistosomal activity in mice at the U. S. Army Medical Research Unit/Brasilia. Our research is aimed towards finding prophylactic and curative drugs for use in the prevention and treatment of schistosomiasis.

PROGRESS:

a. Laboratory Facility. We have upgraded the laboratory equipment giving us limited general laboratory capability by having on hand: 1) portable sterilizer, 2) 12 place centrifuge, 3) constant temperature box, 4) oven, and 5) a lyophilizer.

b. Animal Facilities. Many laboratories throughout the world use a woodchip or sawdust type bedding to house mice. In our early years of anti-schistosome drug testing in Japan, 1964, we had a 30 to 40 percent mouse test group failures in which the mouse infections were not up to the standards set and the entire test group results had to be discarded. Until very recently,

1976, the experimental test group's control infected mice in the primary mortality test system had a 30 percent failure rate for mice exposed to 3,000 or more S. mansoni cercariae but failed to give the LD_{99} by the 30th day of infection. In July 1976, we suspected that the infection failures might be caused by toxic substances present in the woodchip bedding. Our analysis of the woodchip bedding did show that substances were present that affected the infection in mice. During October 1976, we changed over from woodchip to ground corn cob bedding for mouse maintenance. The entire breeding colony at the Bioterio of the University of Brasilia and our drug test animals were placed upon ground corn cob bedding. Since the change in mouse bedding, we have had no test group failures. We now know that of the 20 Brazilian woods tested, at least three are toxic to mice and even man. In our opinion, if schistosomiasis research laboratories are experiencing poor infections in mice, the initial problem to address is the bedding.

c. Snail Colony. The laboratory Biomphalaria glabrata snail colony (Paulista Strain) provides a uniform source of S. mansoni cercariae for infecting mice. The snail colony is responsive to our cercarial requirements, and our daily average population of snails shedding cercariae was 1,318 throughout FY77. We expose individually 400 snails weekly to 8 to 10 S. mansoni miracidia that are recovered from macerated infected livers from the schistosome life cycle mice. During the 42 day incubation period, the snail survival rate was 88 percent and of those surviving snails, 52 percent were isolated as positive cercarial shedding snails. The snail facility's cercarial production is a five million cercariae per week (sufficient cercariae to expose 1,250 mice weekly to 4,000 cercariae.

d. Drug Testing. The 500 to 1,000 mg drug samples received allow us to carry out both prophylactic and curative testing for anti-schistosome activity. We test 50 drugs twice monthly for prophylactic activity in the mouse mortality test system. Drugs are given subcutaneously at 1280 mg/kg on the second day after exposure to 3,000 or more S. mansoni cercariae. In the curative test, 60 drugs are tested twice monthly. Each drug is given subcutaneously at 100 mg/kg for five consecutive days beginning 30-35 days after mice were exposed to 175/200 S. mansoni cercariae.

e. Schistosomiasis. A series of experiments were car-

ried out to standardize the Brazilian Paulista strain of schistosomiasis mansoni in mice for the mortality and the curative test systems. Mice were exposed to seven cercarial doses (500, 1000, 1500, 2000, 2500, 3000, and 3500) with four exposure times (15, 30, 45, and 60 minutes). The mouse deaths were recorded daily. Likewise, mice were exposed to 75, 150, and 225 cercariae for 15, 30, 45, and 60 minute exposures. The infection rates were determined by mouse worm burdens. The preliminary data shows that the death rates and the mouse worm burdens are about the same for the four different exposure times.

f. Operating Personnel. The drug testing program is directed by one American Senior Investigator and supported by a staff of eight Brazilian Laboratory Assistants (one position vacant). The operating program is broken down into five work areas which are: 1) Snail Colony (2 people), 2) Animal Service (2 people), 3) Necropsy (1 person), 4) Pharmacy (2 people), and 5) Administration (1 person). All individuals are cross-trained to perform the seven day work schedule of daily snail maintenance, subcutaneous and gavage drug administration, daily mouse maintenance with mortality checks, and mouse exposures to cercariae. Each individual is able to perform all duties in two other areas of work.

TEST PROCEDURE:

Routinely all drugs received are first screened for prophylactic anti-schistosomal activity by the mouse mortality test system (Radke, et. al., 1971), and secondly some selected and all active drugs are screened for curative activity. The mice used in both test systems are the Swiss-Holland 40 albino mice, 43 $\pm$ 5 days old, weighing 18-23 grams.

The mortality test system evaluates drugs for prophylactic activity. Mice are tail-exposed individually to 3,000 or more S. mansoni cercariae for 45 minutes. Two days after cercarial exposure, the drugs are given subcutaneously in a single inoculation at 1280 mg/kg to five test animals. Active drugs are identified by mouse survival. Each prophylactic test group uses 315 mice; 250 infected mice are used to screen 50 drugs, 50 infected nontreated control mice, five Niridazole treated mice (reference drug), and 10 normal mice. The infected nontreated control mice will begin dying on the 20th day of infection and none will survive

beyond the 30th day in most cases. Active drugs are those in which mice survive two weeks after all infected control mice are dead. At 49 days, all surviving mice are sacrificed and perfused (Radke, et.al, 1961) for total worm burden determination.

The primary curative test (PCT) system as used in the Japan anti-schistosome drug testing program during the years 1968-71 was never published, except for a short description appearing in the WHO Schisto Report (1973.30). This brief account, however omitted critical phases of the test procedure. The PCT uses infected mice that have been tailed exposed for 30 minutes to 200 Schistosoma mansoni cercariae (Puerto Rican strain). Thirty to 35 days postexposure the drugs are administered orally at 100 mg/kg in five daily doses; three days later the treated and untreated infected mice are killed, their livers removed, and liver squash preparations made for worm counts. An active drug in the PCT is identified by an increase in the number of live worms in the liver (more than 10 per mouse) and an increase in the total number of worms in the liver, with the concurrent presence of dead worms. The worm shift is considered to be a result of drug pressure.

When we undertook to expand the Brasilia Anti-schistosome Drug Development Program in 1976 to include a curative test (PCT), we first observed that the reference drug Niridazole had questionable activity against the Brazil Paulista strain of S. mansoni when given orally (100 mg/kg) under the conditions listed above. Secondly, the liver squash worm burden counts from infected control mice were 2 to 3 times higher than the 0 to 5 worms reported from the Japan studies in which the Puerto Rican strain of S. mansoni was used.

A preliminary primary curative test performed at WRAIR, using the cercariae from the same S. mansoni Puerto Rican strain as was used in the Japan anti-schistosome drug testing program, tentatively confirmed the Japan findings of 0 to 5 worms in livers of the infected control mice. However, the infected mice were killed individually by cervical dislocation, livers removed immediately (within 15/20 seconds) and liver squash preparations made for worm counts. A second experiment (Personnal Communications from Reid/Loizeaux, 1977) demonstrated that when mice were killed by cervical dislocation or ether and the livers were not removed for 10 to 15 minutes, then the worm counts from

the squash preparations averaged 12 and 29 worms respectively. We were encouraged by this report, and we discontinued using ether to kill groups of five mice and began to kill mice individually by cervical dislocation followed by the immediate removal of the liver (within 15 seconds) for the liver squash preparation. However, using the above procedure, the liver squash worm counts were more than 10 worms per mouse (range 10 to 30) for 11 of the 15 PCT groups. Our next approach was to investigate worm crowding as a possible factor for the increased numbers of worms recovered in our infected control mice. Three experiments were carried out using mice, tail exposed for 30 minutes to 75, 150, and 225 S. mansoni cercariae. The infected mice were killed as described above and the mean liver squash worm counts per mouse for 150 cercariae exposures were: 10, 12, and 15, and for 225 cercariae exposures were: 13, 10, and 12. In mice exposed to 75 cercariae, the average numbers of worms per mouse from the liver squash preparations were lower (7, 4, and 5). However, in mice exposed to 200 cercariae, the range in total worm burdens (as determined by perfusion) varied from 50 to 100 which was similar for either the Puerto Rican or Brazilian Paulista strains of S. mansoni. Consequently, the primary curative test (PCT) is still under development. Studies are currently underway to determine if the differences in worm numbers in the liver are strain related or possibly are related to other environmental factors. In the Brasilia PCT system, we use as the reference drug Oxamniquine which is given subcutaneously (equally effective orally) at 100 mg/kg for five consecutive days. A potentially active drug is one in which we find a 2 to 3 fold increase in worms in the liver squash preparation with some worms being sick, and/or the presence of dead worms. Such test results are confirmed by a second identical test. A routine primary curative test uses 340 mice; 300 infected mice for screening 60 bottle number drugs, 15 infected mice are treated with Oxamniquine (reference drug), 15 are untreated infected controls and 10 are normal non-infected mice.

RESULTS:

During FY 1977, a total of 2,096 bottle number drugs were tested in either the prophylactic or curative test system. Using the mortality test, 1,310 bottle number drugs were tested either at 1280 mg/kg or at a lower retest dosage of 40 and 160 mg/kg. The prophylactic test results were: 879 drugs were negative,

428 drugs were toxic, and 3 drugs were active (see Figure 1). In the curative test, 786 bottle number drugs were screened for anti-schistosome activity at either 100 mg/kg for five days or at a lower dosage. Drug test results were: 704 negative, 41 toxic, 35 unconfirmed actives that were tested only once, and 4 active (see Figure 1). Only one bottle number drug ZN 07500, a discreet compound, was tested twice in the PMT and PCT systems and found active. The "active" compounds are listed only by code numbers at the request of the Division of Medicinal Chemistry (WRAIR) since many (but not all) are protected proprietary secrets ("commercially discreet").

LITERATURE CITED

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2. Radke, M. G., Berrios-Duran, L. A., and Moran, K. : A Perfusion Procedure (Perf-O-Suction) for Recovery of Schistosome Worms. J. Parasit. 47: 366-368, 1961.

FIGURE 1

A List of Bottle Number Drugs Found to be Active against Schistosomiasis mansoni by the Mouse Mortality Test and Curative Test System

WRAIR/BRASILIA Bn No. Drug	Mortality Prophylactic Test*	Curative Test*
BC 07 271 (BR 1203)		320/400
BC 21 646 (BR 565)D		40
BC 42 618 (BR 1532)D		40
BE 97 551 (BR 1777)D	1280	
BG 52 598 (BR 2079)	1280	
ZN 07 500 (BR 2560)D	160/1280	25/50

* = Confirmed by repeat testing; dosages in mg/kg, administered subcutaneously

D = Discreet Compound

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The University of Brasilia and USAMRU (.WRAIR) Brasilia tested 2,096 bottle number compounds for either the prophylactic or curative activity against schistosomiasis mansoni in mice. In the prophylactic test, three active drugs were identified out of 1,310 bottle number drugs tested. In the curative test, 4 active drugs were identified out of 786 bottle number drugs tested. One drug was active in both the prophylactic and curative tests.

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